

Applicant : Siamak Tabibzadeh
Application No. : 10/014,320
Filed : December 11, 2001
Page 2

Q2 --The *ebaf* (lefty-A) cDNA was originally cloned in a pBluescript^R SK⁻ vector. A forward primer (5'-AGAATTCAAGATGTGGCCCCTGTGGCTCTGCTGGGC-3' - SEQ ID NO:3) and the reverse primer (5'-TTCTAGACTATGGCTGGAGCCTCCTTGGCACGAGCGCCCC-3' - SEQ ID NO:4) were used to amplify the coding region of *ebaf* with the 3' proofreading polymerase, Pfu (Stratagene, La Jolla, CA). The PCR products were separated in 1% agarose gel, and purified with a GeneClean kit (Bio101, LA Jolla, CA). The PCR products and the plasmids (pcDNA3 or HA-pcDNA3) were digested with EcoRI and XbaI (New England Lab, Beverly, MA). The fragments were annealed to a mammalian expression plasmid (pcDNA3 or HA-pcDNA3) with a Rapid Ligation Kit (Stratagene, CA). The sequence of the selected clone was validated by restriction enzyme digestion and by sequencing using Taq DyeDeoxy terminator cycle sequencing reactions in conjunction with an Applied Biosystems model 373 DNA Sequencer. The plasmid DNAs containing the correct cDNA sequence insertions were prepared using the Promega Wizard Miniprep Method (Promega, Madison, WI), and used for transfection.--

Please amend paragraph 71, on page 28, to read as follows:

Q3 --The N-glycosylation site of *ebaf* (amino acid residue 57) was point mutated to "D" using QuikChangeTM 1-Day Site-Directed Mutagenesis kit (Stratagene, La Jolla, CA), following the manufacturer's protocol. The primers were DRTS-F: 5'-GCGTCCGCGACGACGGCTCCGACCGCACCTCCCTCATCGACTCC-3' (SEQ ID NO:5); DRTR-R: 5'-GGAGTCGATGAGGGAGGTGCGGTCGGAGCCGTCGTCGCGGACGC-3' (SEQ ID NO:6). The sequences of all point-mutated clones were determined by Taq DyeDeoxy terminator cycle-sequencing reactions, in conjunction with an Applied Biosystems model 373 DNA Sequencer.--
